Beneficial Effect of a Moderately Energy-Restricted Diet on Fibrinolytic Factors in Non-obese Men

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Impaired fibrinolytic activity has been reported in the elderly and is thought to play a role in the etiology of cardiovascular disease, one of the leading causes of death in most Western countries. Since restriction of energy intake has been demonstrated to act beneficially on the aging process in a variety of species, we studied the effect of a 10-week moderately energy-restricted (ER) regimen (80% of habitual) on plasminogen activator inhibitor (PAI) activity, PAI-1 antigen, tissue plasminogen activator (tPA) activity, and tPA antigen in non-obese, middle-aged men. Moreover, the relationship between these fibrinolytic markers and glucose tolerance was investigated. Weight loss in the ER group (n = 16) was considerable (−7.4 ± 1.7 kg, $P < .001$). Subjects in the control group (n = 8) also lost some weight (−2.1 ± 1.5 kg, $P < .01$). Fasting glucose levels decreased in the ER group (−0.31 ± 0.48 mmol/L, $P < .05$), which was correlated with the extent of weight loss ($P < .01$). Baseline insulin levels at 2 hours after an oral glucose load correlated with baseline PAI activity ($P < .001$) and PAI-1 antigen levels ($P < .001$). PAI activity decreased in the ER group (−2.94 ± 2.90 IU/mL, $P < .001$), particularly in subjects with a high baseline PAI activity (>9 IU/mL). Furthermore, energy restriction led to decreased PAI-1 antigen concentration ($P < .05$), a nonsignificant increase in tPA activity, and a decrease in tPA antigen concentration ($P < .001$). All these changes were more clear in subjects with a high baseline PAI activity. These results suggest that 10 weeks of moderate energy restriction has a profibrinolytic effect in non-obese, middle-aged men, at least in subjects with higher baseline PAI activity (>9 IU/mL). Moreover, in line with the suggestion that high PAI activity goes together with insulin resistance, a relationship between insulin concentration after a glucose load and PAI activity was found.

RESTRICTION OF energy intake, with adequate intake of essential nutrients, is currently the only dietary regimen known to have a beneficial effect on the aging process in rodents, prolonging the lifespan and delaying or preventing the onset of several age-related diseases.[1] The mechanism behind this process is still largely unknown, and much effort is now being directed at unraveling the causal relationship between energy metabolism and the aging process. Since energy restriction has been demonstrated to be effective in a variety of species, these beneficial effects might also hold for human beings on an appropriate energy-restricted (ER) regimen. Obviously, it is difficult to assess the potential effect of energy restriction in human in an experimental design. However, as a first approach, the feasibility and potential health risks/benefits of a moderately ER diet can be assessed. Another approach would be to study the effects of energy restriction on physiological systems associated with the aging process.

Cardiovascular disease is one of the leading causes of death in most Western countries and has been related to the aging process. Impaired fibrinolytic activity has been reported in the elderly[2] and is thought to play a role in the pathogenesis/etiology of cardiovascular disease. Activity of the fibrinolytic system depends on competing processes in a dynamic equilibrium involving circulating activators of plasminogen, primarily tissue-type plasminogen activator (tPA), and circulating inhibitors of these activators, primarily plasminogen activator inhibitor type-1 (PAI-1). Since energy restriction has a beneficial effect on the aging process, it may also have an effect on hemostatic function. Therefore, as part of a study of the potential benefits and/or risks of a 10-week moderately ER regimen in non-obese, middle-aged men, activities and concentrations of indicators of the fibrinolytic system (PAI activity, PAI-1 antigen, tPA activity, and tPA antigen) were analyzed. In addition, since it has been suggested that PAI-1 contributes to the increased cardiovascular risk together with insulin resistance,[3] the relationship between fibrinolytic markers and glucose tolerance was investigated.

SUBJECTS AND METHODS

Subjects

Twenty-four apparently healthy men aged 43 ± 5 years (range, 35 to 50) with a mean body mass index (BMI) of 24.6 ± 1.8 kg/m^2 (range, 20.6 to 27.2) participated in this study. All subjects had a stable weight during the past year and normal routine clinical chemistry and were nonsmoking. They had normal dietary habits and no extreme level of energy intake (9.3 to 13.2 MJ/d, ie, 97 to 214 kJ/kg/d). Each subject was medically screened on the basis of a health questionnaire and a medical examination. Informed consent was obtained from each subject, and the research protocol was approved by the Institute's external Medical Ethics Committee.

Experimental Design

A detailed description of the study has been published elsewhere.[4] In short, habitual energy intake was estimated by means of a 7-day dietary record before the study started. The total experiment lasted 12 weeks. Subjects were randomly assigned to one of two groups: a control group (eight subjects) and an ER group (16 subjects). These groups were matched for age and BMI. During the first 2 weeks of the study (run-in period), all subjects received a weight-maintaining test diet based on the outcome of the dietary record. Since energy intake differs among individuals, subjects were divided into six energy groups with a stepwise increase of approximately 770 kJ/d, ranging from 9,304 to 13,147 kJ/d. When

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subjects lost more than 1 kg/wk during this run-in period, they were reclassified into a higher energy group.

After these 2 weeks, eight control subjects were kept on the weight-maintaining diet for the next 10 weeks, while the other 16 subjects received a diet that contained 80% of the energy of their habitual (weight-maintaining) diet and micronutrient levels of at least the recommended dietary allowances. Energy restriction was achieved by substituting low-fat and artificially sweetened products for ordinary products. This implied that the relative contribution of carbohydrates, fat, and protein to total energy intake was 51%, 34%, and 15%, respectively, for the control group and 47%, 36%, and 17%, respectively, for the ER group. All foods and drinks to be consumed during these 12 weeks were supplied by the Institute. Subjects were not allowed to eat or drink anything but the diet that was supplied (except water).

At the end of the run-in period and at the end of the experiment, blood samples were drawn after an overnight fast for analysis of indicators of the fibrinolytic system and fasting ($t = 0$) glucose and insulin concentrations. Thereafter, an oral glucose tolerance test (OGTT) was performed according to the World Health Organization protocol:[5] 2 hours after an oral glucose load (75 g in 250 mL water, which had to be drunk within 15 minutes), another blood sample ($t = 120$) was drawn for analysis of glucose and insulin concentrations.

Methods

Plasma glucose concentrations were analyzed with a commercially available kit (Boehringer Mannheim Diagnostica, Hitachi, Japan). Plasma insulin concentrations were analyzed with a commercial radioassay (Pharmacia, Roosendaal, The Netherlands) according to the kit protocol.

For analysis of fibrinolytic factors, blood was drawn into ice-chilled tubes from the intermedian cubital vein by a venoject system. After centrifugation for 10 minutes at 1,700 × g and 4°C, the plasma was collected, snap-frozen, and stored at −80°C until analysis. Under these storage conditions, the fibrinolytic factors PAI activity, PAI-1 antigen, tPA activity, and tPA antigen have been shown to be stable for at least 1 year.

To determine PAI activity, PAI-1 antigen, and tPA antigen, blood was collected in CTAD buffer (0.11 mol/L citrate, 15 nmol/L theophylline, 3.7 mmol/L adenosine, and 0.198 mmol/L dipyridamole; Becton Dickinson, Meylon, France). For measuring tPA activity, blood was collected in Stabilyte tubes (Biopool, Umeå, Sweden). PAI activity was measured by a titration method with two-chain tPA according to the method reported by Verheijen et al.[6] Results are expressed relative to pooled plasma: 100% activity corresponds to neutralization of 7.6 IU/mL tPA. PAI-1 antigen level was measured by an enzyme-linked immunosorbent assay (Coalize PAI-1/Innotest PAI-1; Chromogenix, Möndal, Sweden).[7] tPA activity was measured with a bioimmunoassay for tPA (Coatest BIA t-PA; Chromogenix).[8] tPA antigen level was measured with the Imulyse t-PA method (Biopool).

Intraassay variation for PAI activity, PAI-1 antigen, tPA activity, and tPA antigen is 6% to 8%, 4% to 6%, 5% to 7%, and 8%, respectively. Interassay variation for these four measurements is 10%, 6% to 8%, 5%, and 10%, respectively.

Statistics

BMDP statistical software (Los Angeles, CA; version 1990 VAX/VMS) was used to detect changes within and between groups and to calculate correlations between parameters. Changes within groups were tested for significance with the paired Student's t test. Differences in changes between the two groups were tested with the unpaired Student's t test. Relationships between two variables were calculated with Pearson's linear correlation coefficient. Since the control group also lost some weight, correlations were calculated for the entire subject population (N = 24).

RESULTS

Baseline subject characteristics (age, body weight, BMI, and OGTT) and the change (Δ) after the experimental period are presented in Table 1. Both groups lost weight. However, as might be expected, subjects in the ER group lost significantly more weight, resulting in a decrease in BMI. Fasting glucose levels ($t = 0$) significantly decreased in the ER group, with no significant difference between the ER group and the control group. This decrease in fasting glucose level correlated with the extent of weight loss ($r = .531$, $P = .008$) and with the change in BMI ($r = .576$, $P = .003$). With regard to parameters of the OGTT, no other significant changes were observed.

The results on fibrinolytic parameters are presented in Table 2. At baseline, these parameters did not differ significantly between groups. Energy restriction resulted in significantly lower PAI activity. Further inspection of the data (Fig 1) showed that the decrease of PAI activity in the ER group was modest in subjects (n = 9) with baseline PAI activity levels not greater than 9 IU/mL (subgroup 1), but was most marked in subjects (n = 7) with baseline PAI activity greater than 9 IU/mL (subgroup 2). PAI-1 antigen level decreased significantly in the ER group. In this group,

Table 1. Baseline Physiological Characteristics and the Change (Δ) After 10 Weeks of Moderate Energy Restriction

Characteristic	Control Group		ER Group		P (Δcontrol v ΔER)
	Baseline	Δ	Baseline	Δ	
Age (yr)	43 ± 5		43 ± 4		
Body weight (kg)	77.7 ± 6.6	−2.1 ± 1.5†	78.6 ± 9.3	−7.4 ± 1.7‡	<.001
BMI (kg/m²)	24.6 ± 2.2	−0.8 ± 0.6	24.9 ± 1.8	−2.3 ± 0.8‡	<.001
Glucose (mmol/L)					
$t = 0$	4.95 ± 0.44	0.02 ± 0.30	4.87 ± 0.40	−0.31 ± 0.48*	NS
$t = 120$	4.44 ± 0.92	−0.08 ± 0.67	4.44 ± 1.04	0.02 ± 1.38	NS
Insulin (mU/L)					
$t = 0$	8.25 ± 2.82	−0.96 ± 2.38	7.37 ± 2.75	−1.38 ± 3.00	NS
$t = 120$	30.5 ± 19.9	−1.5 ± 6.4	24.9 ± 21.5	−1.0 ± 22.7	NS

*$P < .05$.
†$P < .01$.
‡$P < .001$.

Table 2. Baseline Levels of Fibrinolytic Factors and the Changes (Δ) After 10 Weeks of Moderate Energy Restriction

Fibrinolytic Factor	Control Group		ER Group		*P* (Δcontrol *v* ΔER)
	Baseline	Δ	Baseline	Δ	
PAI activity (IU/mL)	9.3 ± 3.2	0.7 ± 3.6	10.3 ± 4.4	−2.9 ± 2.9‡	.018
subgroup 1	7.4 ± 1.8	−0.7 ± 0.5	7.0 ± 1.5	0.5 ± 0.6*	NS
subgroup 2	12.3 ± 2.6	3.0 ± 5.7	13.6 ± 3.8	−5.4 ± 2.1‡	.007
PAI-1 antigen (ng/mL)	57.1 ± 40.0	−4.6 ± 40.2	60.8 ± 41.3	−30.1 ± 38.8*	NS
subgroup 1	45.9 ± 44.5	−15.3 ± 28.0	39.1 ± 16.1	−18.5 ± 17.3*	NS
subgroup 2	75.6 ± 82.4	13.3 ± 57.8	82.4 ± 48.4	−41.7 ± 51.5	NS
tPA activity (mIU/mL)	291 ± 116	−91 ± 91*	341 ± 247	50 ± 165	NS
subgroup 1	352 ± 89	−100 ± 78	490 ± 268	−8 ± 217	NS
subgroup 2	189 ± 80	−76 ± 127	192 ± 90	108 ± 61†	.012
tPA antigen (ng/mL)	4.9 ± 2.8	−1.1 ± 0.8	6.3 ± 3.5	−3.1 ± 2.1‡	.005
subgroup 1	3.3 ± 1.8	−1.1 ± 1.1	4.4 ± 3.3	2.0 ± 2.4	NS
subgroup 2	7.6 ± 1.7	−1.2 ± 0.3	8.3 ± 2.5	−4.3 ± 0.8‡	.000

NOTE. Subgroup 1, baseline PAI activity ≤9 IU/mL: control group, n = 5; ER group, n = 9. Subgroup 2, baseline PAI activity >9 IU/mL: control group, n = 3; ER group, n = 7.

*$P < .05$.

†$P < .01$.

‡$P < .001$.

the high-PAI subgroup (subgroup 2) showed the strongest decrease in PAI-1 antigen level, but this decrease was not significant ($P = .08$). tPA activity decreased significantly in the control group, but not in the ER group. However, when baseline PAI activity was taken into account, tPA activity increased significantly in subgroup 2. tPA antigen decreased significantly in controls and ER subjects, although the effect was more pronounced in the ER group, especially subgroup 2.

A similar subgroup analysis was also performed for the control group, although the subgroups are relatively small (n = 3 and 5) for statistical analysis.

Correlation coefficients were calculated between baseline values and between changes in the parameters analyzed. Baseline PAI activity was inversely correlated with tPA activity ($r = -.683$, $P < .001$) and was significantly related to PAI-1 antigen concentration ($r = .675$, $P < .001$) and tPA antigen concentration ($r = .640$, $P < .001$). Baseline insulin level at 120 minutes was significantly related to baseline PAI activity and antigen levels. Baseline BMI was not correlated with baseline PAI activity, but the decrease in BMI was correlated slightly but significantly with the decrease in PAI activity (Table 3).

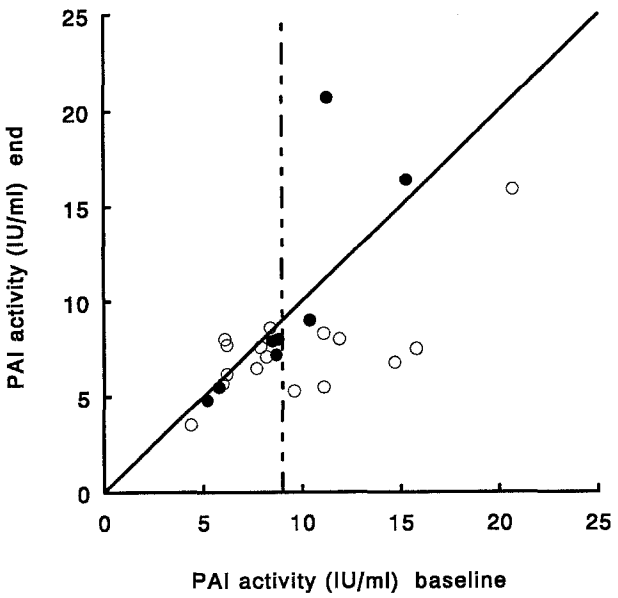

Fig 1. Relationship between PAI activity at baseline and at the end of the intervention period. (○) ER subjects; (●) controls; (-) y = x; (---) PAI = 9 IU/mL.

DISCUSSION

The aim of this study was to examine the potential health risks or benefits of a moderately ER diet, with special regard to fibrinolytic factors. High PAI activity, leading to decreased fibrinolytic activity, can predict coronary thrombosis.[9] There is general agreement on the presence of a decreased fibrinolytic activity in obesity: in observational studies, an inverse relationship between obesity and fibrino-

Table 3. Correlations (*r*) Between Baseline Fibrinolytic Factors and Some Physiolocal Parameters (BMI and OGTT) and Between Changes in These Parameters (N = 24)

Parameter	PAI Activity	PAI-1 Antigen	tPA Activity	tPA Antigen
Baseline				
BMI	.295	.349	−.151	.264
Glucose, *t* = 0	.330	.305	−.296	.339
Insulin, *t* = 0	.291	.226	.053	.394
Glucose, *t* = 120	.504*	.594†	−.245	.360
Insulin, *t* = 120	.711‡	.752‡	−.538†	.619‡
Change				
BMI	.414*	.360	−.330	.442*
Glucose, *t* = 0	.126	.180	−.244	.006
Insulin, *t* = 0	−.008	−.082	.440*	.311
Glucose, *t* = 120	.052	.253	.044	.045
Insulin, *t* = 120	.073	.303	−.084	.016

*$P < .05$.

†$P < .01$.

‡$P < .001$.

lytic activity[10,11] and a positive correlation between BMI and PAI activity[12,13] have been found. From a case control study, it appeared that obese subjects had decreased fibrinolytic activity associated with elevated PAI-1 concentrations, which in turn correlated with increased concentrations of insulin.[14] The small range of BMI in our study probably explains why we did not find an association between BMI and fibrinolytic factors, as compared with the large range (16 to 39 kg/m^2) in previous studies mentioned.

Intervention studies on the effects of energy restriction and/or weight loss on the fibrinolytic system have been reported especially for obese but hardly for lean subjects, and were focused either on very-low-calorie diets or on surgical treatment. In obese subjects (BMI > 30 kg/m^2), very-low-calorie diets for 3 months[15] or 6 months[16] have been reported to decrease factor VII,[15,16] PAI activity,[15] PAI-1 antigen,[16] and tPA antigen,[16] which is associated with marked weight loss. Surgical treatment resulted in a mean decrease in body weight of 64 kg at 12 months, and was accompanied by significant reductions in factor VII, fibrinogen, and PAI activity.[17] From these intervention studies, it can be concluded that fibrinolytic activity increases after severe weight loss in the obese. However, dieting also occurs among lean subjects. Ogston and McAndrew[10] noted that a diet of 590 kJ/d for 3 days increases fibrinolytic activity in non-obese subjects. To our knowledge, no studies have reported on the effects of moderate energy restriction in non-obese subjects. Therefore, this is the first longer-term study into the effects of moderate energy restriction on fibrinolytic factors showing that even moderate energy restriction has beneficial effects on fibrinolytic factors, at least in subjects with higher PAI activity (>9 IU/mL). Although this decrease could be ascribed to a regression to the mean, it is noteworthy that PAI activity did not decrease in the control group (Fig 1).

In the present study, the effect of energy restriction on the lipid profile was also measured, the results of which are presented elsewhere.[4] Briefly, no significant effects of energy restriction on fasting triglyceride, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol concentrations were observed using Student's t test. However, the decrease in body weight was significantly related to an increase in high-density lipoprotein cholesterol. PAI-1 antigen has been associated with several cardiovascular risk factors, including hypertension, hypertriglyceridemia, glucose intolerance, and type II diabetes mellitus.[18] In this study, PAI activity correlated significantly with fasting triglyceride, total cholesterol, and low-density lipoprotein cholesterol concentrations ($r = .743$, $P < .001$, $r = .506$, $P = .012$, and $r = .514$, $P = .010$, respectively, in 24 subjects). In addition, subjects with PAI activity greater than 9 IU/mL (n = 10) did not differ in BMI or in systolic or diastolic blood pressure, but had significantly higher baseline glucose levels at $t = 0$ ($P = .015$) and tended to have higher insulin levels at $t = 120$ ($P = .059$). This association suggests a link between PAI-1 antigen and insulin resistance (ie, "resistance to the glucose-stimulating effects of insulin in key organs such as muscle tissue, liver and fat, leading to higher glucose concentrations, albeit within the normal ranges"[19]). This link has been studied by Potter van Loon et al[3] by means of a sequential hyperinsulinemic-euglycemic clamp study in obese subjects. They found that PAI-1 antigen correlated significantly with peripheral insulin resistance. In our study, significant correlations between baseline fasting PAI activity/antigen and glucose and insulin levels at 2 hours after a glucose load were observed. This finding suggests a similar relationship between insulin sensitivity and PAI activity, even in lean subjects. After 10 weeks of moderate energy restriction, the correlation between PAI activity and insulin at $t = 120$ was less obvious but still significant ($r = .565$, $P = .006$), probably due to smaller ranges.

The mediator between insulin and PAI is not yet clear.[20,21] In vitro secretion of PAI-1 by cultured hepatocytes in promoted by insulin.[9]

In conclusion, this study shows that moderate energy restriction in non-obese men with PAI activity levels greater than 9 IU/mL has beneficial effects on fibrinolytic factors. In addition, before the intervention period, PAI activity was significantly correlated with insulin and glucose levels at 2 hours after a glucose load, in line with the suggestion that higher PAI activity encounters insulin resistance. Since moderate energy restriction has a beneficial effect on the aging process and since impaired fibrinolytic activity is associated with aging, energy restriction might, at least in part, delay the aging process by increasing fibrinolytic activity.

Since this study presents the results of a 10-week ER diet, we can only speculate on the longer-term effects. However, it is difficult to conduct controlled feeding studies for periods longer than 12 weeks. In this study, we aimed at 20% energy restriction, but due to underreporting of habitual energy intake as indicated by weight loss in the control group, the imposed energy restriction was probably higher than 20%. Since subjects were still losing weight at the end of the intervention period with no sign of a tendency to stabilize, we believe that the imposed level of energy restriction might be too high and unfeasible as a long-term intervention. Therefore, in future studies, we will focus on longer-term feasibility of a more moderate energy restriction regimen under free-living conditions. However, the results of this study clearly show that moderate energy restriction is also relevant in lean subjects and has a health-potentiating effect.

Since this study presents only fibrinolytic factors and not true fibrinolytic activity, it would also be worthwhile to study the effect of a period of moderate energy restriction on true fibrinolytic activity. However, from the results of this study, ie, decreased PAI activity and increased tPA activity in the high-PAI subgroup, an increased true fibrinolytic activity might be expected. Since this study shows that moderate energy restriction acts profibrinolytically in healthy non-obese men, it would also be valuable to study whether these results are representative for other populations, including those with higher risks for cardiovascular disease.

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